Transketolase and 2-oxoglutarate dehydrogenase activities in the brain and liver of the developing rat

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Summary. Rat brain transketolase showed little change in activity from birth to adulthood, whereas the liver enzyme activity increased in a biphasic way. In both brain and liver, 2-oxoglutarate dehydrogenase activity increased gradually after birth and reached a plateau at 5 weeks of age. A developmental change in thiamin content in the brain was similar to the change in the 2-oxoglutarate dehydrogenase activity, but this was not the case in the liver.

Key words. Thiamin; 2-oxoglutarate dehydrogenase; transketolase; development.

Thiamin plays a role, in the form of thiamin diphosphate, as a coenzyme of two mitochondrial enzymes, pyruvate dehydrogenase (EC 1.2.4.1) and 2-oxoglutarate dehydrogenase (EC 1.2.4.2), and one soluble enzyme, transketolase (EC 2.2.1.1). In addition, it is considered to have a specific role in the nervous system independently of its coenzyme function, but the details have not yet been clarified[1–4]. On the other hand, neuronal structures undergo biochemical modifications throughout postnatal maturation that are involved in changes in the function of the central nervous system. Thus, a developmental study on brain thiamin metabolism may contribute to clarifying the role of thiamin in the nervous system. It is known that the activity of pyruvate dehydrogenase in rat brain is high after birth[5–7], but there is little information on the other two thiamin-dependent enzymes. In this paper, we determined 2-oxoglutarate dehydrogenase and transketolase activities and thiamin content in rat brain and liver during development, in order to characterize a developmental change in brain thiamin metabolism.

Materials and methods. Sprague-Dawley rats were bred in our laboratory, so that the exact postnatal age was known. The rats were decapitated and their brain cortex and liver were divided into two portions. One was homogenized with 40 mM glycylglycine buffer (pH 7.6) and centrifuged for 1 h at 105,000 × g, and the supernatant was used for transketolase assay. The other was homogenized with 0.32 M sucrose to prepare a crude mitochondrial fraction as previously reported[4]. The mitochondrial fraction was used for 2-oxoglutarate dehydrogenase assay. Transketolase and 2-oxoglutarate dehydrogenase were determined by the methods of Geel and Dreyfus[8] and Holowach et al.[9], respectively. Protein was measured by the method of Lowry et al.[10] using bovine serum albumin as standard. Thiamin compounds were extracted from the tissues with 10% trichloroacetic acid and converted to the fluorescent derivatives. Then they were analyzed by a HPLC system using an ODS-column (Shimpac clc-ODS, Shimadzu, Japan). The details will be published elsewhere[11]. Total thiamin content was measured by this method.

Results and discussion. Figure 1 shows postnatal changes of transketolase and 2-oxoglutarate dehydrogenase activities in rat brain and liver. The transketolase activity in the liver increased stepwise during development, while that in the brain showed little change. The first plateau of the liver enzyme was observed at about 3 weeks of age and the second one was around 5 weeks. On the other hand, the brain and liver 2-oxoglutarate dehydrogenases showed a similar increase after birth. Figure 2 shows the development of thiamin content in the brain and in the liver. The developmental pattern of brain thiamin content was different from that of liver thiamin content; the content in the brain increased until 5 weeks, when it reached a plateau, while that in the liver reached a maximum at 2 weeks and decreased soon afterwards.

The rats took mother milk until 3 weeks after birth, and then they were weaned. This suggests that the type of feeding is involved in an increase of rat liver transketolase activity after 3 weeks. In this connection, a preliminary experiment has

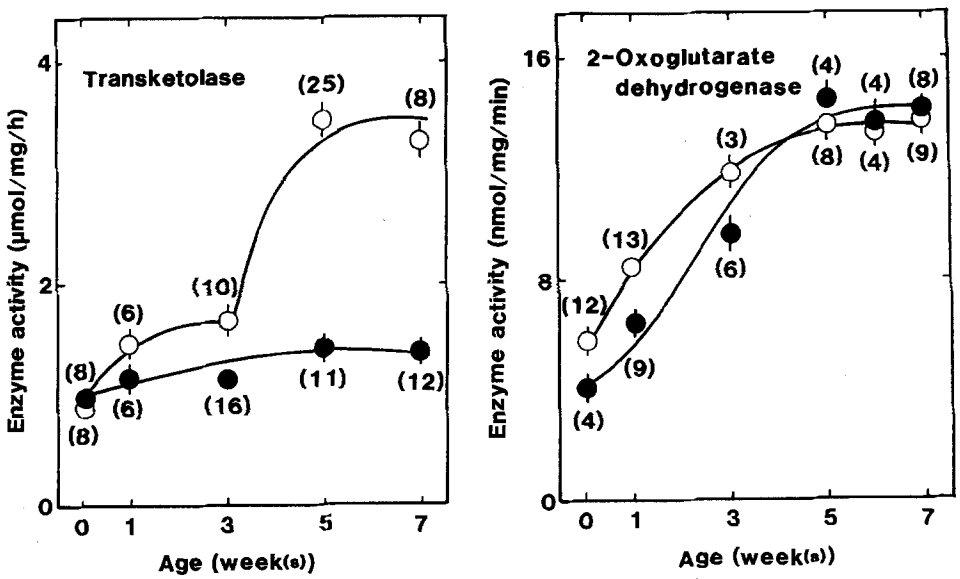

Figure 1. Postnatal development of transketolase and 2-oxoglutarate dehydrogenase activities in rat brain (●) and liver (○). The results shown as μmol/mg protein of the soluble fraction/h for transketolase and nmol/mg protein of the mitochondrial fraction/min for 2-oxoglutarate dehydrogenase are mean ± SEM of the number of experiments shown in brackets.

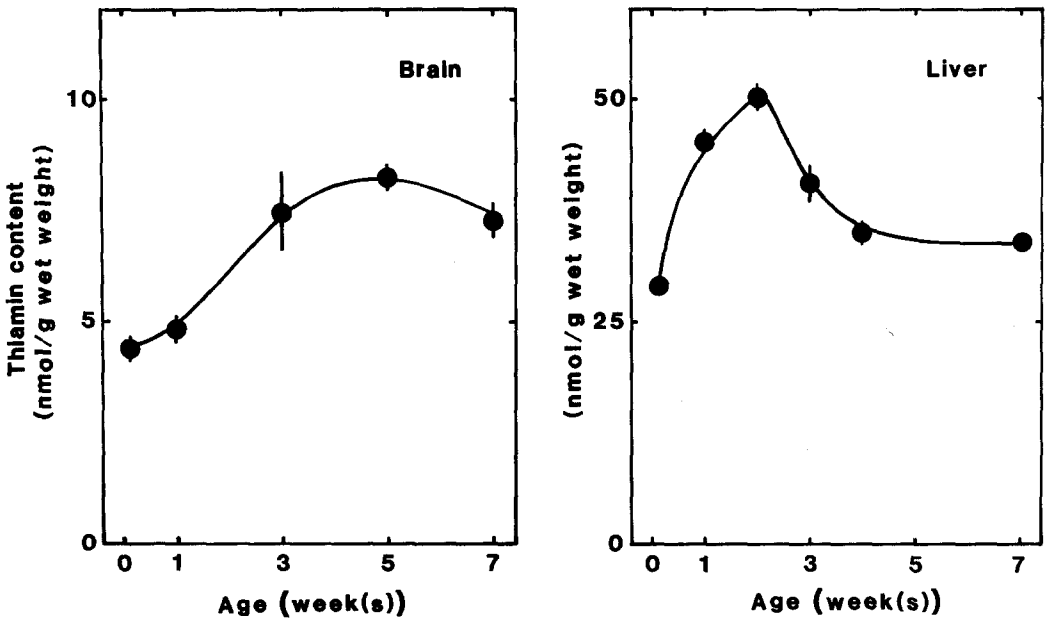

Figure 2. Postnatal development of thiamin content in rat brain and liver. Each point is mean ± SEM of 3–4 determinations.

shown that the increase is blocked by pretreatment with actinomycin D (0.4 μg/kg, i.p., 3 days, in 3-week-old rats), suggesting that synthesis of the enzyme is induced at the weaning period. Since the development of the brain transketolase was different from that of the liver enzyme, there might be differences between the brain and liver in the activity of the hexose monophosphate pathway during development. The low activity of adult rat brain transketolase seems to be consistent with the previous finding in developing chick brain that the activity of the hexose monophosphate pathway is high during embryonic life, then gradually declines at the time of hatching, and almost completely disappears in the adult [12].

It is known that a number of mitochondrial enzymes including pyruvate dehydrogenase and mitochondrial protein increase during development [5–7]. Land et al. [7], however, reported that the development of rat brain citrate synthase, a mitochondrial enzyme, preceded that of brain pyruvate dehydrogenase by about 1 week and that the pyruvate dehydrogenase activity was still increasing after the development of both citrate synthase and mitochondrial protein had ceased. Though we did not assay other mitochondrial enzyme activities in this study, the developmental patterns of the brain 2-oxoglutarate dehydrogenase and thiamin content in the brain were similar to that of pyruvate dehydrogenase as reported by Land et al. [7]. However, the developmental changes in the brain 2-oxoglutarate dehydrogenase activity and thiamin content seemed to be different from those in other mitochondrial enzyme activities and mitochondrial protein. These findings, together with the previous results [5–7], suggest that brain thiamin metabolism may change during development, although the activity of transketolase, a thiamin-dependent soluble enzyme, only changed slightly during development. This assumption might be related to the previous observation [8] that developing rats were much more susceptible to thiamin deficiency than adults. The present findings also suggest that the activities of thiamin-dependent mitochondrial enzymes such as pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase in the brain may reflect the thiamin level in the tissue. In contrast, postnatal changes in liver thiamin content were more complex, and the activities of thiamin-dependent enzymes in the liver were not directly related to thiamin content in the tissue. Thus there might be a difference between the brain and the liver in the development of thiamin metabolism. In recent separate experiments, we also found a difference between brain and liver in the postnatal development of the enzymes involved in thiamin metabolism (unpublished).

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